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NEW OBSERVATIONS ON COLLEMBOLA MIDGUT

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The ultrastructure of the collembolan midgut is well known from to the work of Dallai (1966), Krzysztofowicz _et al_. (1973), Humbert (1974), Dallai and Callaini (1979), Eisenbeis and Meyer (1986) and Barra and Poinsot Balaguer (1987). These studies have demostrated that the collembolan midgut is without peritrophic membrane; that at its posterior end there are not Malpighian tubules which are replaced by a "pyloric ring"; that it is responsible for the main excretory functions.

The availability of simple new techniques with specific markers for the distribution of cytoskeletal elements, permitted investigations of the precise organization of the visceral muscles surrounding the midgut and the observation of new cytological features.

MATERIAL AND METHODS

Midguts of _Hypogastrura meridionalis_, _Onychiurus armatus_, _Folsomia multiseta_, _Proisotoma granulata_, _Cryptopygus bipunctatus_, _Isotomurus palustris_, _Orchesella cincta_, and _Dicyrtomina ornata_ were studied. These specimens were dissected in 0.1 M pH 7.2 cacodylate buffer or in pH 6.8 phosphate buffered saline (PBS) or in 0.1 M pH 7.2 Na phosphate buffer (Na B).

Conventional electron microscopy (TEM). The dissected midguts were fixed in a 4% paraformaldehyde and 5% glutaraldehyde mixture (Karnovsky, 1965) in cacodylate buffer plus 5% sucrose for 1 h, then washed overnight with the same buffer and postfixed with 1% OsO_4 in cacodylate buffer for 1 h. After rinsing with cacodylate buffer, the material was dehydrated and embedded in Epon-Araldite. Thin sections, routinely stained, were observed with a Philips 400 EM.

Heavy meromyosin (HMM) treatment. Midguts, dissected in Na phosphate buffer, were transferred to 0.3 mg/ml saponine in the same buffer for 5 min at room temperature. After rinsing in Na phosphate buffer the material was treated with 50% glycerol in the same buffer for 4 hours at 4° C, then with 2.5 mg/ml S_1 myosin fraction in 50% glycerol (kindly provided by Dr. Alan Weeds, Cambridge) in Na phosphate buffer for 16-18 hours at 4° C. Fixation was carried out for 30 min at room temperature in 1% glutaraldehyde in Na phosphate buffer to which 2 mg/ml tannic acid and

6% saccarose were added. The material, carefully washed with distilled water, was postfixed at room temperature with 0.50% osmium tetroxide in distilled water for 30 min. Specimens were rinsed in distilled water and stained en bloc in 2% aqueous uranyl acetate for 30 min. The material was dehydrated, embedded and sectioned as previously described and observed with a Philips 400 EM.

Epifluorescence microscopy. Midguts collected in pH 6.8 PBS were fixed for 20 min with 4% freshly prepared paraformaldehyde in the same buffer and then transferred for 10 min. to the same solution containing 0.1 Triton X-100. The midguts were throughly rinsed in PBS and incubated for 20 min with 1.5 μg/ml phalloidin labeled with rhodamine (Molecular Probes inc.). The material was rinsed in PBS and mounted in 90% glycerol containing 2.5% n-propyl gallate to reduce photobleaching (Giloh and Sedat, 1982).

Fluorescence observations were carried out with a Leitz Aristoplan microscope equipped with rhodamine filter. Photomicrographs were taken with Kodak Tri-X pan film.

Scanning electron microscopy (SEM). For scanning observations the gut was critical point dried in a Balzer apparatus. After coating with gold, the material was observed with a Philips 501 SEM.

OBSERVATIONS

The collembola midgut is a cilynder (0.25 mm in diameter and about 2 mm long in Orchesella villosa) which extends for most of the body length. Posteriorly it is continuous with the hindgut which is about half the length of the midgut. The midgut is surrounded by an orderly network of intrinsic visceral striated muscle fibers which can easily be reconstructed in detail in SEM preparations (Pl. I, a,b) or using material previously treated with rhodamine-labeled phalloidin, which is specific for actin filaments (Pl. I, c). The longitudinal muscle fibers, about 20 in number, surround the midgut; they seem to terminate in the pyloric region, where they anastomose with hindgut muscles. Beneath these longitudinal fibers a series of transverse circular muscle fibers encircle the gut. There are about 100 of these fibers spaced 15-20 μm apart. In the posterior part of the gut they are closer because of contraction. Careful examination of these circular fibers shows that at two opposite points along their length, each fiber divides into two branches (Pl. I, c); one of these branches joins one of the adjacent fibers. Thus circular muscle fiber consists of two semicircles, which are not in perfect continuity but slightly mutually displaced.

This complex and precise organization of the two types of muscle fibers is shared by all the species of collembola examined, although the number of fibers constituting the muscle meshwork enveloping the midgut may vary.

The material treated with rhodamine-labeled phalloidin also shows two opposite longitudinal series of clusters of fluorescent dots (Pl. I, d). Each cluster, which is located in the space originating from the crossing of longitudinal and circular muscle fibers, contains 30-50 fluorescent dots.

To understand the origin of these fluorescent dots we observed thin sections by TEM. Cross sections through the basal epithelial region showed fat-body cells with finger-like prolongations that make contact with the basal part of the epithelial cells (Pl. II, a). These prolongations, in the shape of large polylobate structures, penetrate into the epithelial cells, pushing the basal lamella inward. These prolongations are filled with 5-6 nm thick microfilaments which also continue into the cytoplasm of the fat-body cell. It is difficult to ascertain how many clusters belong to a single cell~~s~~; we saw up to three projections per cross section, each corresponding to a single dot (Pl. II, a).

The midgut has cells with 2.2 µm long microvilli, containing a bundle of longitudinal microfilaments (Pl. II, b). More than 10-15, 5 nm thick filaments form a core in each microvillus. They usually penetrate the apical cytoplasm for about 0.6 µm giving rise to a fibrous layer beneath the apical plasma membrane. The portion of a microfilament in the apical cytoplasm has been called the rootlet.

This aspect is shared by all collembolan species examined so far, irrespective of the family to which they belong. The only exceptions are a few genera of Isotomidae, namely *Isotoma* and *Isotomurus*. As first described by Dallai and Callaini (1979) and more exhaustively by Eisenbeis and Meyer (1986) and Barra and Poinsot Balaguer (1987), these species have a belt structure surrounding the cell about 0.7 µm from the apical membrane. In TEM cross sections this structure, which is 0.24 µm thick, receives microfilaments from microvilli and consists of packed filamentous material (Pl. II, b). The belt structure contains 0.20-0.26 µm in diameter pores, the edges of which are denser than the neighbouring area. The distance between two adjacent pores varies from 0.15 to 0.30 µm. Vesicles, short endoplasmic cisterns and microtubules can pass through the pores from deep in the cytoplasm towards the cell apex. Mitochondria, multivesicular bodies and lamellar structures cross the pores less frequently, even though they have been observed to do so. The belt structure makes lateral contact with material adhering to the plasma membrane involved in the organization of the smooth continuous junction (Pl. II, b).

If the midgut epithelium of *Isotomurus* is treated with rhodamine-labeled phalloidin a continuous fluorescent apical band is evident (Pl. I, e). The height of this band seems to correspond to the brush border of the microvilli (Pl. I, e-f). Because of the vicinity of the belt structure to the apical plasma membrane, it is quite difficult to establish whether or not the fluorescence also involves the belt. The absence of fluorescence in midgut cells during the renewal process, when microvilli are lacking but the belt-structure still persists, suggests

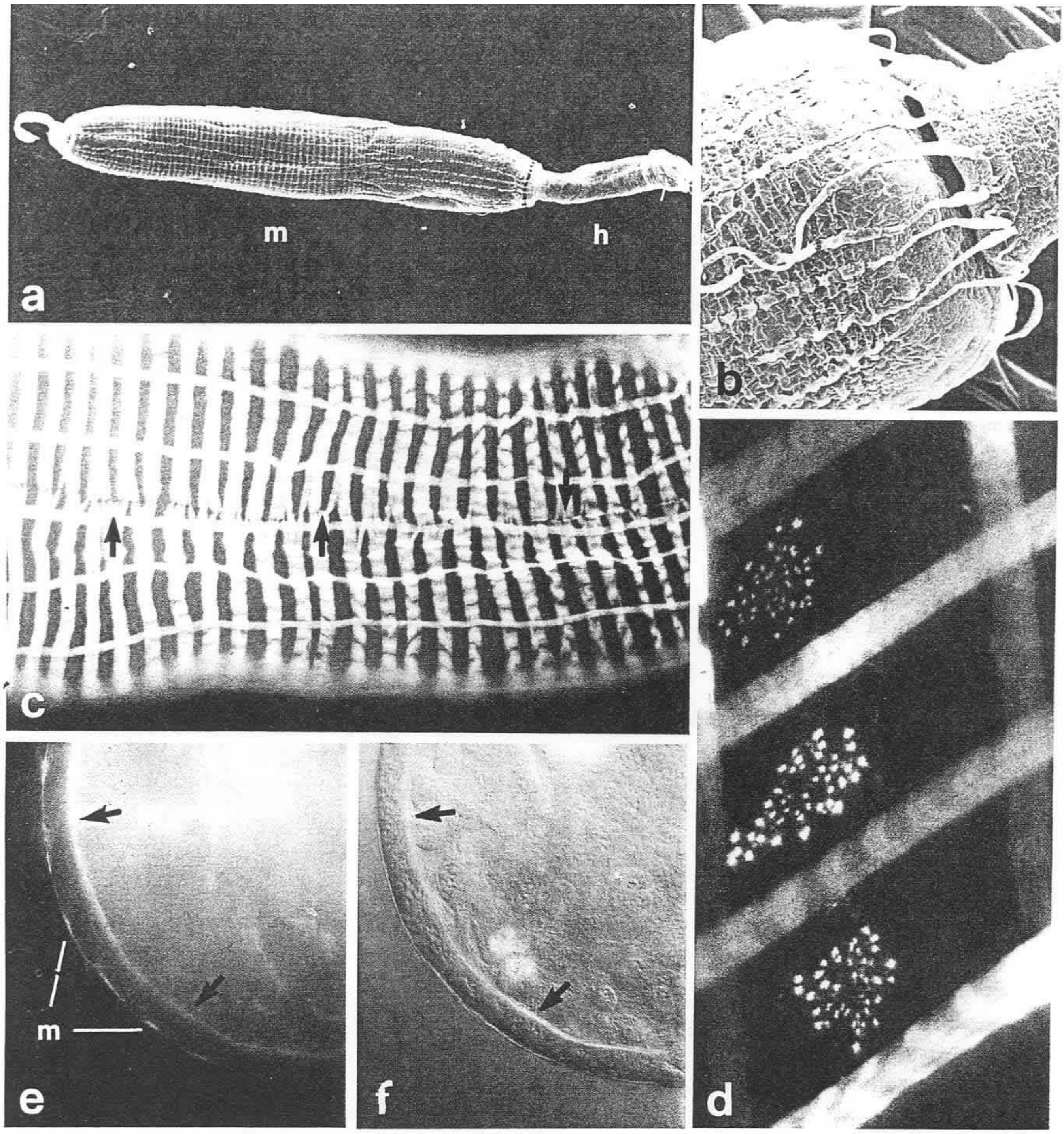

Plate I. a) SEM micrograph of the gut of Orchesella villosa. m, midgut; h, hindgut; x 40. b) Detail of the piloric region; x 250. c) Whole mount of the midgut of O. villosa treated with Rh-phalloidin for actin filaments. Circular muscle fibers branch in the dorsal and ventral regions (arrows); x 250. d) Particular of three clusters of fluorescent dots, visible in the lateral regions of the midgut of O. villosa; x 500. e) and f) Sagittal view of the midgut wall of Isotomurus palustris. The brush border (arrows) is stained by Rh-phalloidin (arrowheads). m, longitudinal muscle fibers; x 250.

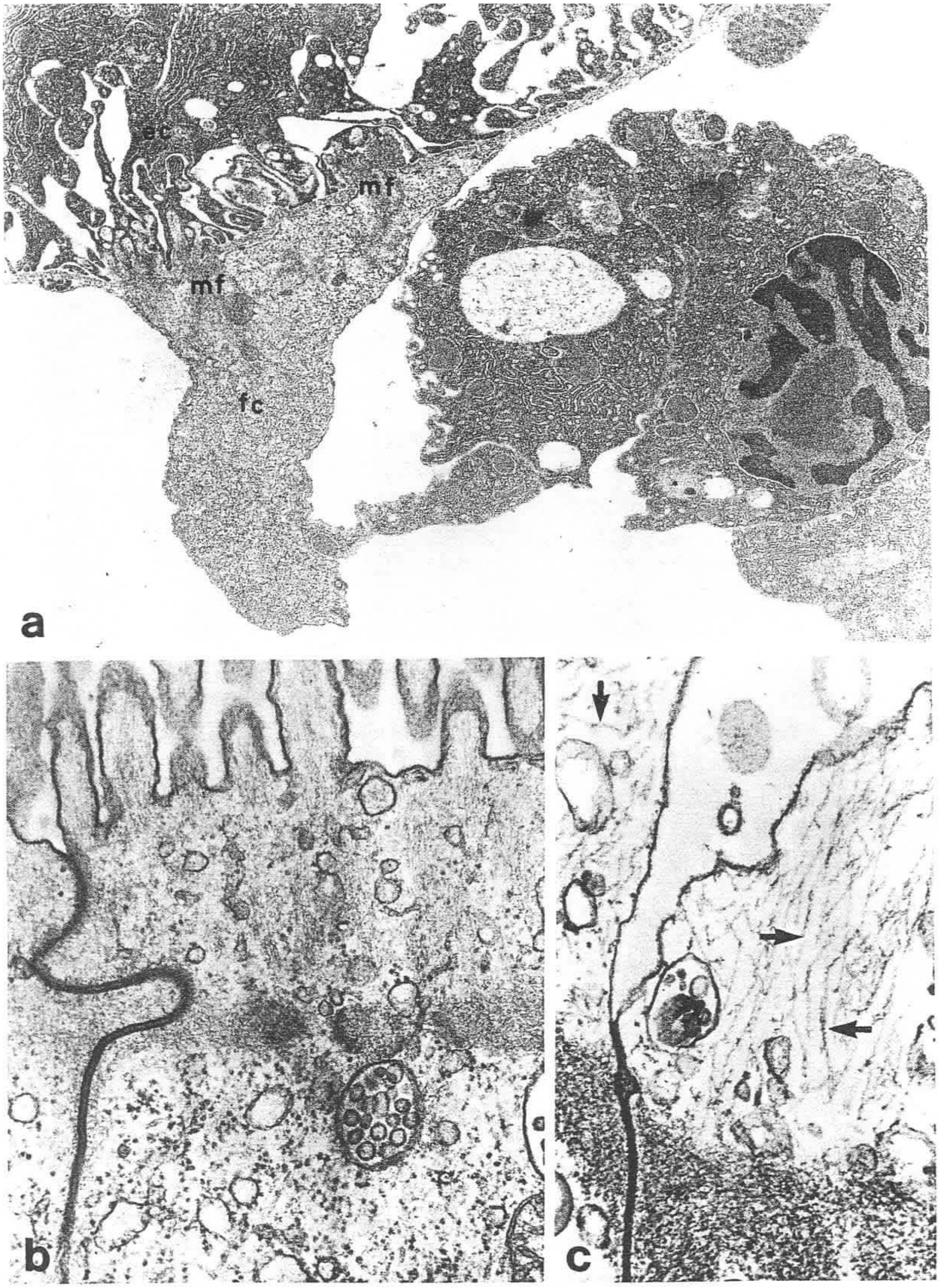

Plate. II a) Thin section of the basal region of the midgut of Orchesella villosa showing fat-body cells (fc) wich make contact with the epithelial cells (ec). mf, microfilaments. x 10000. b) and c) Detail of the apical epithelial region of the midgut of Isotomurus palustris. Microvilli are filled with thin microfilaments wich continue in the cytoplasm. The microfilaments form arrowhead complexes with S_1-myosin fragment (arrows).

that actin-like material does not occur here.

Confirmation of this idea comes from experiments with the S_1-myosin fragment to decorate actin filaments present in the cell. A decoration clearly appears on microfilaments in the apical region of the cell where there are swallen microvilli (Pl. II, c). Decoration indicates that actin filaments are oriented down towards the belt structure; on the contrary decoration does not seem to occur in the material of the belt structure Pl. II, c).

DISCUSSION

The study of different collembolan species provided further information on the structural organization of the midgut.

1. The intrinsic visceral muscle complex surrounding the midgut consists of circular and longitudinal muscle fibers disposed regularly to form a regular meshwork. The organization is constant in the different species examined and seems to repeat the typical pattern of other insects (Chapman, 1985, for a review).

2. Two lateral strands of fat-body run along the midgut, tightly adhering to the epithelial cells through prolongations from the fat-body cells, which contain actin filaments.

3. The organization of the intestinal brush border is well known in vertebrates (Mooseker, 1985, for a review). According to the nomenclature proposed by Hull and Staehelin (1979) and then by Mooseker (1985) the apical cytoplasm of the cell, into which the core rootlets of the microvillar microfilaments descend, is known as the terminal web. This region can be divided into an apical zone, an adherens zone and a basal zone, the latter two characterized by the association of microfilaments and tonofilaments with the belt and spot desmosomes respectively. In the inter-rootlet zone there is a meshwork of fine, non- actin, filamentous material which interdigitates, and presumably cross-links, adjacent core rootlets. Two chemically distinct classes of filaments have been detected: myosin and spectrin (Hirokawa, 1982; Hirokawa _et al_., 1983; Glenney _et al_., 1983; Pearl _et al_., 1984).

We have no information about the terminal web of invertebrates, insects included. The only available data is that of Kukulies _et al_. (1984), showing fluorescent microfilaments in the apical region of the midgut cells of dragonfly and that of Lane and Flores (1988) on the association of actin fibrils with the membranes of septate junctions. On the other hand, even the organization of insects and collembola midgut cell junctions is different from that in vertebrates. Midgut cells are only joined by a continuous junction (Noirot and Noirot, 1967; Dallai, 1975), which extends down for a long distance. No other specializations, such as _zonula_ and _macula_ _adhaerentes_, occur along the cell contact. This apparently more simple organization in insects and collembola cannot easily be likened to that described in Vertebrates. The pattern described in Cryptopygus, Isotoma and Isotomurus, the only isotomid species with an apical belt structure (Dallai and Callaini, 1979; Eisenbeis and Meyer, 1986; Barra and Poinsot, 1987) is peculiar to insects and collembola and appears to be the only pattern reminiscent of vertebrates, even without the complex junctional specializations of this group. In this respect the term "terminal web" used by Dallai and Callaini (1979), if extended to the whole apical cell region, seem appropriate and underlines the similarity with the pattern in the vertebrate midgut.

We do not want to discuss the suggestions of Eisenbeis and Meyer (1979) and Barra and Poinsot (1987) about the functional meaning of the terminal web. It should be remembered that pinocytosis and periodic renewal of the midgut are common traits of all collembolan midgut cells, as is the ingestion of mineral bodies. Hence it seems untenable that the terminal web is associated with these processes. It certainly cannot be said that the terminal web is specific to the whole Isotomidae; it is actually only peculiar to the genera Cryptopygus, Isotoma and Isotomurus. The chemical composition of the belt structure is not yet known. Our preliminary experiments with rhodamine-labeled phalloidin and with the S_1-myosin fragment indicate that the belt structure does not contain actin, whilst decorated actin filaments are clearly evident in the apical cell region oriented down towards the belt structure. The use of antibodies specific for other cytoskeletal elements, has given negative results (data not published), but it was expected because of the specificity of the markers.

Experiments are in progress to isolate the belt structure and ascertain its biochemical characteristics. Only with these data will it be possible to establish the nature and function of this interesting structure.

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